denoted as TC association ($4.7 \pm 0.3 \times 10^7$ $M^{-1}s^{-1}$) and EF-Tu dissociation (12 ± 1 s^{-1}), are found with Cy3-Cy5 FRET pairs or Cy3-QSY9 pairs placed on L11/EF-Tu, tRNA/EF-Tu (A-site) or tRNA/tRNA (A-site/P-site) pairs. The reaction rates are almost independent of labeling strategy and agree with ensemble measurements. At 10 ms time resolution, the FRET between L11/EF-Tu and tRNA/EF-Tu (A-site) pairs showed only one EF-Tu bound conformational state that is detectable during the tRNA selection process, providing strong evidence that, at this time resolution, EF-Tu loses proximity essentially simultaneously with both L11 and aa-tRNA. Alternating-laser excitation experiments demonstrate that, following EF-Tu dissociation, a fraction of aa-tRNA remains stably bound to the ribosome, corresponding to aa-tRNA that has successfully accommodated into the A-site, while the remainder dissociates rapidly, presumably due to rejection via proofreading.

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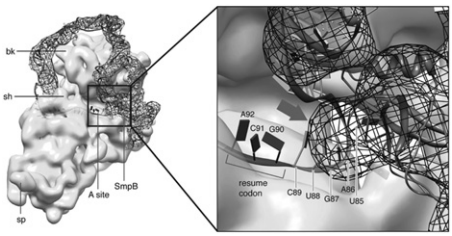
2190-Pos Board B176

The First Atomic Model of the Transfer-Messenger RNA in the Resume State

Yaser Hashem, Jie Fu, Joachim Frank.

Bacterial ribosomes stalled by truncated mRNAs are rescued by transfer-messenger RNA (tmRNA), a dual-function molecule (362 nucleotides in *E. coli*) that contains a tRNA-like domain (TLD) and an open reading frame (ORF) carried on an mRNA-like domain (MLD). The tmRNA, in association with a small protein (SmpB), enters the ribosome, occupies the empty A site with its TLD bound to the SmpB, and switches the translation to its own ORF that codes for a degradation tag. In this study, based on our cryo-electron microscopy (cryo-EM) density map and biochemical data, we used homology/*ab initio* modeling techniques to build the first detailed atomic model of the tmRNA in complex with the *E. coli* 70S ribosome in the ''resume state,'' where the TLD and the SmpB are at the P site, and translation has resumed on the ORF. Here, we describe how the atomic model was constructed based on experimental data, and how it sheds the light on the trans-translation resuming mechanism implicating the correct reading frame selection on the tmRNA and the passage through the ribosome, considering its complex topology.



Exocytosis & Endocytosis

2191-Pos Board B177

Energy of Formation of a Clathrin Coated Pit

Anand Banerjee, Ralph Nossal.

The assembly of a clathrin coated pit (CCP) during endocytosis is a highly cooperative process that requires the spatial and temporal coordination of several constituents. The kinetics of CCP assembly is not well understood, but it is clear that the energy of creating a high curvature bud from a plasma membrane of much lower curvature plays a crucial role. We present a phenomenological model for the energy of formation of a CCP and express the energy in terms of the size and curvature of the pit. Our model contains three terms, viz., the energy needed to bend the plasma membrane, a line tension energy, and the energy stored in chemical bonds. We show that for reasonable values of the parameters involved in the problem, the plot of the free energy of a CCP with size shows an energy barrier which has to be crossed in order for a growing CCP to transform into a vesicle. We discuss the sensitivity of the energy barrier to the various attributes of the model.

2192-Pos Board B178

Coarse-Grained Simulations of Membrane Remodeling by Bar Domains

Haosheng Cui, Edward Lyman, Gregory A. Voth.

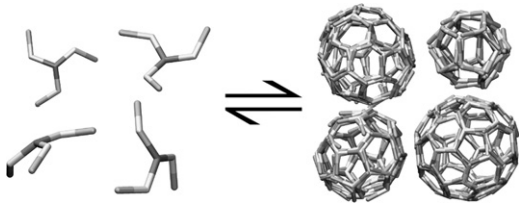
Endophilin is a critical protein involved in clathrin mediated endocytosis. The N-BAR (N-terminal helix and Bin/amphiphysin/Rvs) domain of endophilin interacts directly with the lipid membrane, binding to regions of highly curvature at the neck of endocytic vesicles. Experiments have shown that the N-BAR domain tubulate the membrane and forms a lattice-like oligomer coat on the membrane tubule *in vitro*. The ordered oligomer structure may account for the observed N-BAR density on the membrane, which is important for dynamin recruitment. Achieving a high resolution picture of the oligomer structure experimentally is challenging, especially for the part of the protein that interacts with the membrane, such as the N-terminal amphipathic helices. Novel coarse-grained (CG) simulations are therefore reported that facilitate a deeper understanding of these oligomer structures. A CG model, guided by both atomistic molecular dynamics simulation data and cryo-EM (cryo-electron microscopy) images, can be constructed. Screened electrostatic and dipole-dipole interactions in the CG modeling are used. Multiple oligomer structures are simulated and compared, which are guided by reconstruction of cryo-EM images. Large-scale CG simulations, consisting of ~500K CG sites for the phospholipid and proteins, are also performed to study the early stages of membrane remodeling of liposomes induced by endophilin N-BAR domains.

2193-Pos Board B179

Clathrin Self-Assembly into Polyhedral Cages by Computer Simulations

Wouter K. den Otter, Marten R. Renes, Wim J. Briels.

Clathrins are three-legged proteins that self-assemble into polyhedral cages with important regulatory and mechanical functions in the formation of cargo-laden vesicles at the cell-membrane (endocytosis) and the trans Golgi complex. The essential features of self-assembly are innate to clathrins, as cages are also formed in purified slightly acid solutions. This bare-bones process has been simulated here for the first time, revealing that an asymmetric distribution of interactions along the leg's circumference, rather than clathrin's characteristic shape, holds the key to the self-assembly process. The global puckering of the triskelion determines the average cage size, while the distribution of pentagonal and hexagonal facets in the self-assembled cages follows a simple selection rule. Simulations of planar clathrin lattices indicate that the introduction of spontaneous curvature, through a change of the clathrins's puckers, does not make a plaque curl up into a cage, but instead the plaque releases dome-shaped fragments which may subsequently grow into cages by recruiting cytosolic clathrins.



2194-Pos Board B180

Theoretical Modeling of the Weaving of Clathrin into Nanoscale Baskets

Shafigh Mehraeen, Nicholas Cordella, Jee Soo Yoo, **Andrew J. Spakowitz**.

Many biological systems are capable of spontaneously assembling a diverse set of molecular architectures from a single subunit, without the need to pre-pattern the assembly. Cellular uptake of external substances is accomplished by a highly adaptive endocytosis process that accommodates a wide range of cargo shapes and sizes. Clathrin-mediated endocytosis involves the formation of a pit that is surrounded by a honeycomb coating whose pinwheel-shaped subunit is a clathrin-protein complex. We develop a theoretical model for the thermodynamics and kinetics of clathrin assembly, addressing the behavior in 2 and 3 dimensions, relevant to membrane and bulk assembly, respectively. The clathrin triskelions are modeled as effective flexible pinwheels that form leg-leg associations and resist elastic deformation. Thus, the pinwheels are capable of forming a range of ring structures, including 5-, 6-, and 7-member rings that are observed experimentally. Our theoretical model employs Monte Carlo simulations to address thermodynamic behavior and Brownian dynamics simulations to track the motion of clathrin pinwheels at sufficiently long time scales to achieve complete assembly. With this theoretical model, we predict the phase diagram for clathrin assembly incorporating binding interactions, elastic deformation, and defect-pair coupling, utilizing Kosterlitz-Thouless theory of defect-induced melting in 2 dimensions. Using analytical theory and computational simulations, we explore the role of binding strength and clathrin elasticity in the ability for clathrin lattices to dynamically reorganize due to local changes in membrane elasticity and tension. We then proceed to discuss the dynamics of lattice reorganization during the process of a clathrin-coated membrane wrapping around a nanoscale cargo.

2195-Pos Board B181

Rotational Motions of Endocytic Cargos Revealed by Single Particle Orientation and Rotation Tracking (Sport)

Ning Fang, Gufeng Wang, Wei Sun.

Single-particle tracking (SPT) is a powerful approach to probe biological processes at molecular level in live cells. However, conventional SPT techniques are still having difficulties in reporting the orientation and rotational